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REVIEW

Mitocentricity

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Abstract—Worldwide, interest in mitochondria is constantly growing, as evidenced by scientific statistics, and studies of the functioning of these organelles are becoming more prevalent than studies of other cellular structures. In this analytical review, mitochondria are conditionally placed in a certain cellular center, which is responsible for both energy production and other non-energetic functions, without which the existence of not only the eukaryotic cell itself, but also the entire organism is impossible. Taking into account the high multifunctionality of mitochondria, such a fundamentally new scheme of cell functioning organization, including mitochondrial management of processes that determine cell survival and death, may be justified. Considering that this issue is dedicated to the memory of V. P. Skulachev, who can be called mitocentric, due to the history of his scientific activity almost entirely aimed at studying mitochondria, this work examines those aspects of mitochondrial functioning that were directly or indirectly the focus of attention of this outstanding scientist. We list all possible known mitochondrial functions, including membrane potential generation, synthesis of Fe-S clusters, steroid hormones, heme, fatty acids, and CO_2. Special attention is paid to the participation of mitochondria in the formation and transport of water, as a powerful biochemical cellular and mitochondrial regulator. The history of research on reactive oxygen species that generate mitochondria is subject to significant analysis. In the section "Mitochondria in the center of death", special emphasis is placed on the analysis of what role and how mitochondria can play and determine the program of death of an organism (phenoptosis) and the contribution made to these studies by V. P. Skulachev.

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INTRODUCTION

This issue is dedicated to the memory of Vladimir Petrovich Skulachev, who, even after his death, was rated as the no. 1 biochemist in Russia (https://research.com/scientists-rankings/biology-and-biochemistry/ru). He was a biochemist not only by education, but also by style and implementation of thinking. His fundamental understanding of the course of biochemical processes in a living cell has placed him among the most important scientific people in the world. This man has placed the study of mitochondria at the center of his scientific existence. That is why our work has been called "Mitocentricity", which justifies the actions of a great scientist who devoted his life to the study of this unique structure.

If we begin to analyze in detail all possible metabolic pathways in which mitochondria participate, then placing them in some center of biological action

Abbreviations: ROS, reactive oxygen species.

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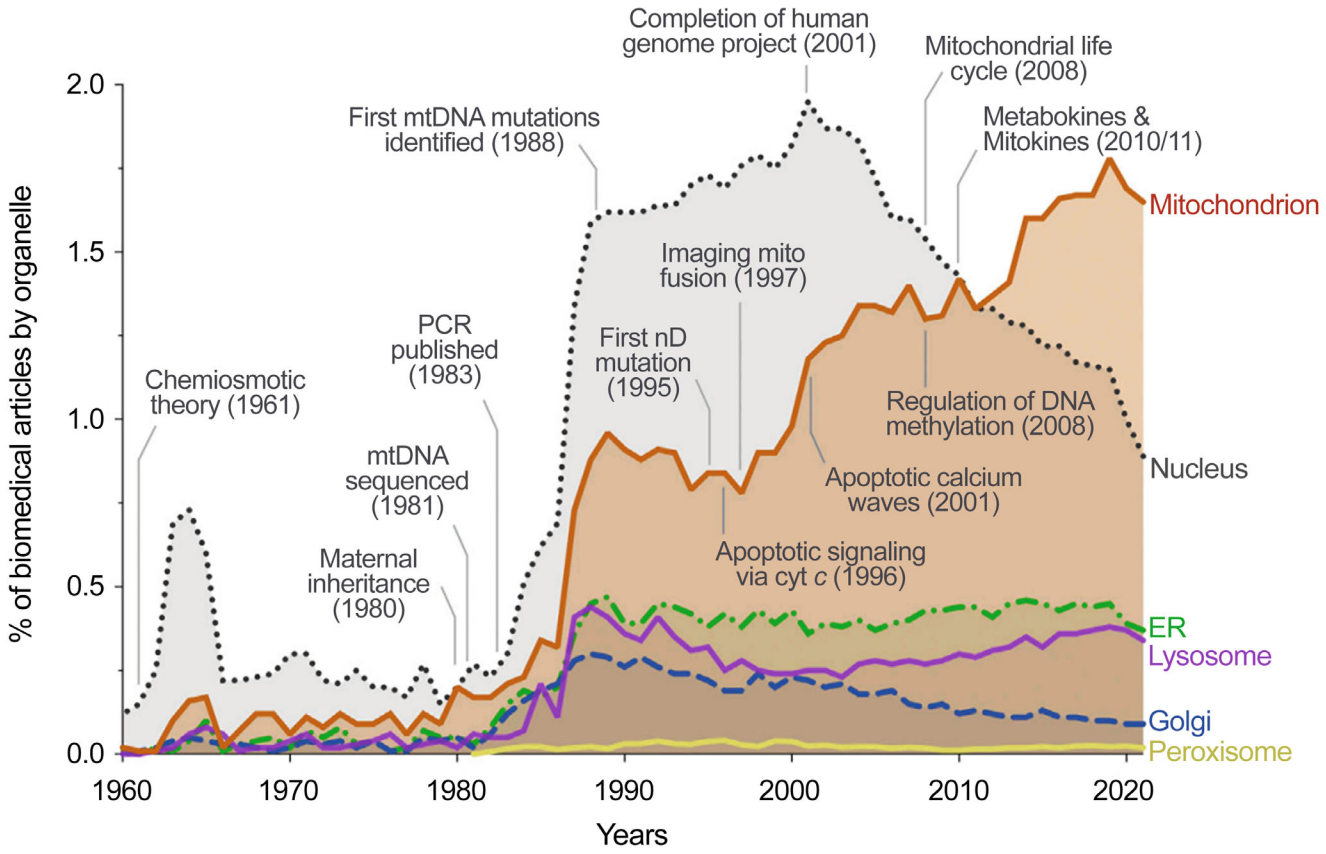

Distribution of publications of biomedical profile concerning the structure and function of various organelles. We note two sharp increases in publications on mitochondria in 1988 and 1996 after publications on the first identified mutations in mitochondrial DNA and participation in programmed cell death, respectively. We also note the decline in publications on the cell nucleus after the completion of the human genome project (from [2] with permission).

seems justified [1]. Given the great number of published works on the activity of mitochondria, we limit our consideration to their role in two polar states – life and death. Therefore, we have divided this work into two parts, one of which will be called “Mitochondria at the Center of Life” and the other “Mitochondria at the Center of Death”, of course, bearing in mind that in the latter case we will talk about the role of mitochondria in the preparation and implementation of the termination of the biological system, while the first part, which is presented in more detail, the role of mitochondria in enabling the vital activity of this system will be presented. This scheme corresponded to the scientific views of V. P. Skulachev, and we will analyze some of their aspects.

We must admit that the placement of mitochondria in the center of biological actions is also objective, which, in particular, is due to the fact that of all cellular organelles, scientific interest to mitochondria does not decrease, but rather increases, while interest to other organelles, firstly, remains significantly lower, and, secondly, either it does not change much over the years, or it shows a negative trend, as in the case of nuclear research (figure). The figure shows that in 2022 almost 2% of all biomedical publications were dealing with mitochondrial activity.

One can only assume that such interest is caused by the demonstration of the polyfunctionality of this organelle [3]. It should be noted that throughout almost the entire 20th century mitochondria were considered almost exclusively as energy producers, and only their bioenergetic function was presented in the textbooks available at that time. The enumeration and summation of all possible (in particular, alternative to bioenergetic) mitochondrial functions was largely revolutionary. In terms of the evolution of the scientific view of the role of mitochondria in the cell, there has been a transition from the idea of the monofunctionality of this organelle to its multifunctionality. Moreover, in modern literature the energetic aspects of mitochondrial activity are being considered less and less, bringing to the fore the structural and functional organization of mitochondria. It should be noted that in the works of Vladimir Skulachev, some of which we will discuss below, there was a transition in time from consideration of purely bioenergetic aspects of mitochondrial activity to considering alternative mitochondrial functions of biomedical importance.

It becomes clear that mitochondrial polyfunctionality is most likely explained by the origin of mitochondria. Admittedly, mitochondria originate from certain gram-negative bacteria, which by definition must be multifunctional, which determines the self-sufficiency necessary for survival in an environment characterized by significant chemical and physical diversity and variability.

The transfer of bacteria into a certain eukaryotic prototype, the internal environment of which is characterized by a relatively guaranteed uniformity of composition and conditions, was accompanied by the loss of a number of functions inherent in bacteria. In particular, there was a loss of the flagellum necessary for movement and a number of properties uncharacteristic of the bacterium were acquired (for example, the acquisition of thermogenic function). One can only assume that such interest is caused by the multifunctionality of this organelle [3]. Mitochondria in the process of evolution acquired an adenine nucleotide translocator (ANT), the existence of which in bacteria would be not only meaningless, but also fatal, because all the ATP synthesized in the cell would be released into a dimensionless space. If the point of view of the bacterial origin of mitochondria is true, then some apparently absurd antagonism between mitochondria and other cell elements becomes clear, which will be discussed later.

MITOCHONDRIA AT THE CENTER OF LIFE

Let us consider the basic, often unique, vital functions inherent only in mitochondria, while we will not dwell on those aspects that have been widely discussed in the scientific field.

It should be noted here that the bioenergetic function of mitochondria does not belong to the unique intracellular functions, because there is anaerobic, non-mitochondrial energy production.

Generation of the transmembrane potential of hydrogen ions. The work of some transmembrane enzymes may be accompanied by asymmetric charge separation, which, for example, occurs when the Na,K ATPase located in the plasma membrane normally transfers $3Na^+$ in exchange to $2K^+$ [4] or the already mentioned ANT, catalyzing the exchange of intramitochondrial ATP^{4-} for extra-mitochondrial ADP^{3-} [5]. The same principle of charge asymmetry generation is applicable to mitochondrial proton pumps, which create the transmembrane potential of hydrogen ions, an integral part of which is the electric potential on the inner mitochondrial membrane (minus inside). We have repeatedly pointed out the highest importance of the mitochondrial membrane potential, which is clearly not limited only to support of ATP synthesis in the ATP synthase complex [6]. It would not be redundant to note once again that even in critical conditions, when the generation of membrane potential due to the operation of proton pumps is halted (for example, in hypoxia), the mitochondria uses either a reversed ATP synthase system (mitochondrial ATPase as part of complex V) [7] or coupled activation of the fumarate reductase pathway to create the membrane potential [8, 9].

Maintenance of the membrane potential in conditions unfavorable for oxidative phosphorylation, that is, in conditions of suboptimal energetics, outwardly seems unjustified, incomprehensible and conditionally selfish. However, we assume that the decisive role is played by the need to preserve the high quality of mitochondria, the control of which requires a membrane potential, with the help of which low-functional mitochondria are selected [10]. The criterion of this high quality is the presence of high membrane potential in mitochondria.

The mandatory requirement of a membrane potential for the transport of proteins into the mitochondria [11], which are mostly synthesized outside the mitochondria, can also at least partially explain the requirement of the need for a membrane potential for the general existence of both the mitochondria and the cell. It is significant that homeostasis of the mitochondrial membrane potential is a prerequisite for the healthy existence of mitochondria and host cells, and those mitochondria that do not meet this requirement are subject to disposal by mitophagy [10].

The membrane potential is the driving force of the transport of cations, in particular calcium ions, which play an extremely important role in regulating the metabolism in mitochondria and cells [6]. It is mainly due to the accumulation of Ca^{2+} in mitochondria through the electrogenic Ca^{2+} uniporter that mitochondria are considered as an intracellular buffer of calcium ions on a par with the endoplasmic reticulum [12, 13].

Quantification of the membrane potential in mitochondria requires various kinds of controls, while the most widely used approach is the use of penetrating cations, which have long been called “Skulachev ions”, giving a credit to the founder of this approach [14, 15]. In the section related to the generation of reactive oxygen species (ROS) in mitochondria, we will disclose the role of membrane potential homeostasis in maintaining ROS homeostasis, as one of the most significant discoveries of Skulachev.

Synthesis of iron-sulfur clusters. Iron-sulfur compounds are an essential component of the biological system [16]. Perhaps they are the oldest direct precursors of life – at least so says Wächtershäuser who hypothesized that early life was formed on the surface of minerals containing iron sulfide [17, 18].

Iron-sulfur clusters are universal protein prosthetic groups with different stoichiometric ratios of iron

and sulfur atoms and perform multiple functions in biological systems. They are most often involved in a variety of redox reactions, while their participation in electron transport, ribosome biogenesis, DNA replication and repair, transcription and translation, and several other processes is known [1]. It is assumed that the assembly of the Fe–S cluster was an essential cause of the emergence of mitochondria as an endosymbiont [19, 20], since the synthesis of Fe–S clusters is necessary for the survival of eukaryotic cells [21-23].

Synthesis of steroid hormones. Mitochondria provide the synthesis of some of the most powerful cellular regulators, namely, steroid hormones due to the cleavage of the cholesterol side chain, which occurs with the participation of one of the isoforms of cytochrome P450 (P450ssc). This process takes place in steroidogenic cells of the adrenal glands, gonads, placenta, and brain [24]. An important role in this process is played by the so-called a "peripheral" benzodiazepine receptor (in modern classification called tryptophan-rich-sensory protein (TSPO), located in the outer mitochondrial membrane, which is homologous to the CrtK protein, a tentative oxygen sensor in the cells of *Rhodobacter* bacteria [25, 26].

CO_2 generation. Carbon dioxide (CO_2) is formed in the matrix of mitochondria in the Krebs cycle from isocitrate and α-ketoglutarate, forming carbonic acid, dissociated to form a proton and a carbonate anion. It should be kept in mind that the higher the activity of the Krebs cycle, the more actively the Krebs cycle is functioning (and, correspondingly, the higher the respiration rate), the more CO_2 is formed and the greater is the probability of acidification of the mitochondrial matrix (for more information, see [27]). Besides the fact that the carbonate ion plays a very important role in the homeostasis of cellular pH, it carries important signaling functions [27-33].

Heme synthesis. One of the most important components of redox reactions is heme, which is part of hematoporphyrins and is synthesized in the mitochondrial matrix to provide intermediate reactions in the cytosol [34-36].

The starting substrate of the heme synthesis cascade is succinyl CoA, and the final product is protoporphyrin IX, into which the ferrochelatase enzyme introduces iron ions, completing the synthesis of iron derivatives of heme, particular representatives of which are cytochromes and hemo- and myoglobin. Given the high gross content of heme iron derivatives in higher animals, they are an important reservoir of oxygen and iron in the organism.

Synthesis and utilization of fatty acids. In his works, V. P. Skulachev paid considerable attention to the relationship between mitochondria and long-chain fatty acids. It should always be kept in mind in any consideration of general medical issues, for example, related to the problem of obesity, that both synthesis and β-oxidation of fatty acids occur in mitochondria [37]. It is also necessary to understand that the synthesis of fatty acids starts with acetyl CoA, while the synthesis competes with the non-enzymatic process of acetylation of biological components, as a result of which the synthesis of fatty acids to some extent prevents the accumulation of acetyl CoA and, accordingly, hyperacetylation. The same thing applies to the process of fatty acid oxidation, the activity of which can seriously affect the level of acetyl- and other fatty derivatives of CoA and indirectly and directly affect the acylation process, contributing to the enzymatic and non-enzymatic modification of biological structures.

In his numerous studies, Skulachev pointed out that the level of fatty acids in the cell will largely determine the efficiency of mitochondrial energetics, given the fact that fatty acids are uncouplers of oxidative phosphorylation [38-41].

Water formation and redistribution. In this section, we want to touch in sufficient detail a question that is extremely rarely raised in the scientific community, namely, the issue of water formation in the cell as one of the important regulatory mitochondrial functions. Although mitochondrial activity is usually judged by the level of oxygen consumption, we want to draw attention to the fact that mitochondria consume the bulk of the oxygen taken by cells, mainly associated with the formation of water and carbon dioxide, and this is an extremely important mitochondrial function.

Superficially, it seems that the need to maintain water homeostasis is not a very important problem, but this is not so, because edema of organs (primarily lungs or brain) is ultimately the principal cause of the death of the organism, although edema is not a direct consequence of mitochondrial dysfunction. Therefore, we will review some aspects of the formation and redistribution of water in the cell.

Almost all the food consumed as a result of its utilization by the body turns into water and carbon dioxide (later, when considering the functioning of mitochondria during detoxification, we will discuss the fate of nitrogenous compounds, which will complement the entire set of products formed as a result of food consumption). So, the main product of the oxidation of reduced equivalents as a result of the functioning of the respiratory chain is water, which is formed in the active center of cytochrome oxidase facing the intermembrane space (that is, towards the cytosol) [42]. However, it must be recognized that the calculation of water formation solely basing on oxygen consumption is not correct, since part of the oxygen consumed is at least temporarily used to form oxidized lipids, proteins, and nucleic acids, which is especially important under conditions of oxidative stress.

The reaction of water formation from $2H^+$ formed as a result of proton pumping and an oxygen atom receiving two electrons from cytochrome oxidase is a first-order reaction in oxygen. Thus, the level of the latter will determine the water flux formed in mitochondria. This means that the higher the respiration rate (coupled or uncoupled with ATP synthesis), the higher the water production, and under uncoupled respiration, the rate of water formation will be significantly higher than in the coupled system. This also means that the uncoupled oxidative phosphorylation will lead to a sufficiently strong generation of water in the mitochondria, causing the need to eliminate it from the mitochondria, and then the cell, in order to prevent swelling of the mitochondria and cells. On the other hand, it must be realized that under conditions of hypoxia (physical or chemical latter caused by inhibitors of the components of the respiratory chain, primarily cytochrome oxidase, for example, by carbon or nitrogen monoxides), the rate of water formation in mitochondria due to oxidation will be lower than under normoxia.

On the other hand, we admit that the chemical reaction of ATP synthesis from ADP and P_i is also associated with the formation of water, and it will be higher with the increase of the rate of ATP synthesis, which occurs in the active center of ATP synthase facing the mitochondrial matrix. Thus, it is easy to see that in mitochondria, depending on internal needs and environmental factors, processes of water formation and utilization related to energetics occur, which require maintaining some optimal water homeostasis to solve specific energy problems, ultimately expressed in the problem of maintaining the ratio of delivery to use (supply–demand problem) [43-46]. Admitting that the main energy processes occur in the inner membrane and the mitochondrial matrix, it becomes clear that the degree of matrix hydration is crucial and is one of the key factors in regulating the mitochondrial energetics. It should be noted that energy-dependent structural transitions in mitochondria, accompanied by changes in their volume, were the subject of Skulachev's research in the 70s of the last century [47, 48].

Comparison of the electron-microscopic data with the light scattering one using the suspension of isolated mitochondria, alternative options were suggested for either drying of the matrix (the so-called condensation observed both in state 3 according to Britton Chance and at the first stage of uncoupling) [48, 49-53], or its acute watering. The latter (previously we called this condition mitochondrial edema) is called high-amplitude swelling [54], and it is usually an indicator of the onset of the point of no return, as a result of induction of nonspecific permeability followed by the release of proapoptotic factors from mitochondria that bring cell death closer [55, 56]. It is in the range between these polar states that structural changes in the mitochondria occur in the cell, associated with either mobilization or inhibition of energy metabolism.

The history of studying water transport in mitochondria is quite long, but it cannot be considered successful. This topic has been the subject of study by the classics of bioenergetics, including Lehninger [57], Green et al. [58], Hackenbrock, and others [49, 59].

The failure was largely due to the fact that mitochondria were recognized as imperfect osmometers [60, 61], and the described facts of water distribution in mitochondria did not fit into the laws of distribution in accordance with osmotic and oncotic forces.

According to the calculations of Srere [62], mitochondrial water can exist in a quasi-crystalline phase and, given the very high concentration of ions, small and large protein molecules and nucleic acids in the cytosol or matrix of mitochondria, in principle, each molecule, in particular a macromolecule, can be considered as a crowder, followed by the application of the principle of molecular crowding to the state of the water around these proteins and at a distance [63]. Calculations show a fairly large contribution of inaccessible water to its total concentration, which makes it very difficult to determine available, that is, metabolically active water, since considering all water as a bulk phase is not correct. As noted by Garlid [61], who demonstrated the distribution of uncharged substances over two phases of water in the mitochondrial matrix, one of these phases was osmotically inactive and had a more or less constant volume determined by hydration, and the other was osmotically active, and it can be called volumetric water. An osmotically inactive compartment differs from bulk water in its solvent properties, so that some solutes are excluded and some are preferably dissolved.

Volumetric rearrangements occurring in mitochondria with changes in physiological load change the nature of molecular crowding, in particular for a well-developed model of changes in the conformation of nucleic acid depending on a number of environmental factors, which include changes in the nature of hydrogen bonds, stacking interaction of bases, changes in conformational entropy, changes in the concentration of surrounding counterions and the degree of hydration [63]. In general, all these principles can be applied to molecular crowding of protein molecules [62-66]. This must be taken into account not only to evaluate the available water, which determines the structure and function of proteins in a natural environment with a high concentration of macromolecules, which may strongly depend on dilution, but also to interpret the data in conditions of studying the behavior of isolated mitochondria *in vitro* with the usual use of dilute solutions as an incubation medium. It has been reported that the mitochondrial matrix contains 0.272 μl/mg of water (out of a total amount of 0.555 μl/mg), which

does not respond to osmotic forces, which means that almost half of the water in the matrix is bound and osmotically inactive [67].

NMR relaxation data for a 20% protein solution gave three different values: 10^{-12}, 10^{-9}, and 10^{-3}, which were explained by the existence of three types of water (type I – water in the bulk phase; type II – bound water; type III – non-rotating bound water) [68], and these values can be applied to estimates of the state of water in biological samples. While 20% of the protein contains about 90% type I water, 10% type II water and about 0.1% type III water, a 50% protein solution (which is close to the protein content in the mitochondrial matrix) contains 15-30% bound water (type II). The two types of bound water are characterized by ordering (structuring) depending on the distance from the interphase, unlike bulk water, which is disordered and in which mainly metabolic processes occur [68, 69]. All these calculations and assumptions demonstrate the importance of even small volume changes closely related to the percentage of metabolically active water capable of moving in mitochondria and the cell, as opposed to "abnormal" (using Garlid's terminology [61]), i.e., osmotically inactive, bound water.

Substances causing moderate swelling of mitochondria (Hoe694, Diazoxide, DUDLE, etc.) led to an increase in the volume of mitochondria of cardiomyocytes by only a few percent (up to 5), while the respiration rate of these cells increased by 1/3 [70]. If we take into account the data of Srere [71] that the volume of water in the mitochondrial matrix is less than half of the total volume of the matrix (the rest is occupied by proteins), in which the proportion of metabolic water is unknown, then even assuming that the entire pool is metabolic water, the volume of the matrix in this case increases by a multiple of these experimental few percents, which is equivalent to a very significant change in the volume of the matrix. If this is the case, then as a result of such a small change in the volume of the mitochondrial matrix, the activity of the Krebs cycle, one of the main elements of mitochondrial bioenergetics, may change dramatically [72-74]. This indicates the presence of non-linear relationships between the degree of generation and intake of water into the mitochondria and changes in energy production.

Several physical and related biochemical factors specifically change with changes in the volume of the matrix and surrounding membranes. We can name just a few of them.

1. Changes in the compartmentalization of proteins in the *matrix*, leading to the formation and disintegration of supercomplexes [67, 75-79].

2. Concentration or dilution of metabolites, cofactors and inhibitors of endogenous enzymes [80, 81].

3. A change in the curvature of lipids located in the bends of mitochondrial crystae, which leads to a change in the oligomerization of *membrane* proteins and their kinetic constants [67, 80-83].

4. Changes in the activity of protein kinases depending on the volume in which they are located [84].

However, it should be noted that most calculations of water parameters in mitochondria relate to isolated mitochondria, the configuration of which (a strongly expanded intermembrane space and a condensed matrix) differs from the configuration of mitochondria *in situ* (an expanded matrix and a narrow intracrystae and intermembrane space), which makes calculations of changes in the volume of the mitochondrial matrix very difficult to carry out.

The inability to distinguish between water reservoirs in the cell and mitochondria, while these reservoirs are involved in transport and catalysis in different ways, and, on the other hand, the mismatch of mitochondrial configurations under *in vitro* and *in situ* conditions has become a matter of deep disappointment and has led to a slowdown in some research in this area. Given the great complexity described above in interpreting the mechanisms of changes in mitochondrial volume and related metabolism, a number of assumptions have arisen about the active transport of water into the mitochondrial matrix, supported by certain contractile proteins located inside or attached to the outside of the mitochondria [85, 86]. For example, Lehninger, studying the mechanisms of mitochondrial swelling and contraction, revealed in mitochondria an undialyzable thermolabile factor (contractile factor, C-factor, reviewed in [57]), which is released from mitochondria incubated in a medium with reduced glutathione (it is also released from mitochondria treated by ultrasound). The addition of this factor to the suspension of swollen mitochondria in the presence of glutathione (GSH) and ATP caused their contraction. However, there were strong arguments in favor of excluding such a possibility, based on the fact that the energy required to pump water from the matrix would require much more than can be obtained during metabolism [87]. Alternative mechanisms have been proposed, in particular, combining the immobilization of solutes in the matrix and the participation of internal hydrostatic pressure to offset the influence of osmotic pressure [87]. Other potential elements involved in violating the osmotic laws of water redistribution in the matrix were called structural elements of the matrix, which make it possible to organize a sufficiently rigid intramitochondrial skeleton that does not allow abrupt volume changes without their destruction. This is confirmed by many examples of local rather than global matrix swelling (which again supports the claim that the mitochondria is not an ideal osmometer) [88], as well as early data on the presence of some elements of the intramitochondrial skeleton obtained in electron microscopic experiments [89].

The above rather lengthy discussion of the movement of water between the mitochondrial matrix and the cytosol does not answer the question: which route does water use to enter and leave the mitochondria, and if there are many of them, which of the routes provide the main movement of water.

A new type of mitochondrial bioenergetics has recently been described, as a result of which, due to abundance of potassium ions over hydrogen ions in the cytosol, ATP synthesis by mitochondrial ATP synthase can be achieved by transferring potassium ions in the ATP synthase complex from the cytosol to the matrix [90, 91]. Theoretically, the transfer of osmotically active potassium ion associated with the operation of the ATP synthase complex can also be associated with the transfer of water into the mitochondrial matrix. It is assumed that in this way water molecules can be transported into the matrix, making a certain contribution to the total water content in the mitochondrial matrix. There are at least two circumstances that confirm this postulate. First, X-ray diffraction analysis of the ATP synthase complex revealed water molecules in the c-subunit of ATP synthase, which can participate in the transport of both protons and potassium ions [92, 93], and, secondly, general mechanisms of coupled transport of ions and water through small channels were described [94-98]. The assessment of the effect of each component capable of transferring water in and out of the mitochondria will be directly related to the identification of a mechanism for adaptation to the energy loads of the biological system in order to ensure a balance between energy supply and energy demand.

Generation of reactive oxygen species. It can be agreed that one of Skulachev's strongest achievements was the discovery of the so-called "Skulachev ions" together with E. A. Liberman [14], however, he himself was a vivid apologist for the role of ROS in the vital activity of biological systems and put this part of science in the first place for himself.

The first work on this topic was published by Skulachev in 1995 in the Russian journal *Molecular Biology* [99], and it was assumed that non-phosphorylating oxidation enables a low probability of ROS formation in mitochondria. The idea was outwardly very simple – when an electron moves along the respiratory chain (or any other chain, even not associated with the final consumption of oxygen by cytochrome oxidase), the faster the electron runs, the less likely it is that in a halfway to the final consumer this electron will reach a molecule of free oxygen, eventually forming a superoxide anion. It is clear that in bottlenecks, where there are actually sufficiently high levels of stationary reduced intermediate components of the electron transport chain, this probability increases sharply, which implies a recommendation to minimize the presence of "bottlenecks" in order to prevent unwanted leakage of electrons to oxygen. These bottlenecks in the terminology of one of the founding fathers of the world bioenergetics, Britton Chance, were called "cross-over points" [100] and if within the neighboring components of the transfer chain one is reduced and the other is oxidized, this means that communication between these components is difficult, and this pair of carriers reflects the limiting step in the electron transfer chain. As a result, at these cross-over points three ADP phosphorylation control loci were localized, determining the mechanism of respiratory control. In its absence (in the case of uncoupling of oxidative phosphorylation) from the ATP synthesis, bottlenecks in the respiratory chain disappear, and the generation of ROS in these bottlenecks becomes minimal.

Later, this idea was generalized, and it became the main guide for Skulachev's works, advocating the need to combat excessive generation of ROS [101]. It should be noted that at the very beginning it was quite extreme and generally interpreted as pathogenic any generation of ROS except that which participates in the anti-pathogen protection organized by NADPH oxidase which participates in phagocytosis [102]. Mitochondria have been declared the "dirtiest place in the cell" to be cleared of ROS that cause oxidative changes, including age-related damage [103]. Subsequently, the offensive tone was softened based on the understanding that ROS, in addition to the pathogenic role, also play an important signaling role [104].

The very first fundamental works that presented the meaning and danger of ROS generation in a living system were published in the 40-50s of the last century [105, 106]. Even then, the principles of the work of antioxidants were formulated with a proposal for their practical use to protect against oxidative damage. In 1956, Harman hypothesized the predominant role of ROS in the aging process, which sounds as a free radical (sometimes incorrectly called mitochondrial) theory of aging [107]. This theory has become an integral part of a more global network theory of aging, according to which aging is indirectly controlled by a network of cellular and molecular defense mechanisms, including various anti-stress reactions [108] with the later development and isolation of the inflammatory theory of aging [109-113].

The free radical and inflammatory theories of aging overlap on the basis of the role of mitochondria in aging. In the first mitochondria are considered the main place of organization of oxidative stress, and according to the second, mitochondria are the key place of organization of the inflammatory principle [114, 115].

Self-regulation of ROS production and energy metabolism. Mitochondria have been declared generators and internal regulators of ROS levels in the cell [116]. As for the generation of ROS, it is often consid-

ered that mitochondria are the main generator of ROS in the cell. However, this is not the case, and although mitochondria are a powerful producer of ROS, they are not their main generators. The misconception that mitochondria are the main site of ROS generation was refuted in the Britton Chance group [117] with a full description of the contribution of all cellular components to ROS generation, among which peroxisomes occupy the top line of the list.

It should be noted that the most cited work of V. P. Skulachev was the establishment of a nonlinear relationship between the magnitude of the membrane potential and the generation of ROS in mitochondria [118]. This led Skulachev to come to the conclusion that it is necessary to mildly uncouple oxidative phosphorylation in order to prevent hyperpolarization of mitochondria and avoid undesirable hyperproduction of ROS [15, 39, 40, 119-121].

Detoxification. Usually, the detoxification function realized in mitochondria is limited to the synthesis of urea, as a process of eliminating the decomposition products of nitrogen-containing compounds from the biological system. The two initial stages of the urea cycle (also called the ornithine cycle) take place in the mitochondrial matrix and the cycle ends with reactions occurring in the cytoplasm. The main source of nitrogen bases is the product of deamination, ammonia, which is eventually converted into urea, excreted from the organism by the kidneys and which largely serves as an indicator of the degree of normal renal functioning. However, the detoxification process can be considered more broadly, including biologically active substances in the list of removal of undesirable agents, which must perform their function in a certain range of low concentrations, preventing their excess. Any excess of the concentration of active substances can be fraught with situations leading to the occurrence of pathologies. As a simple example, we can consider molecules of extracellular glutamate, which plays the role of a neurotransmitter in the brain, but only in acceptably low concentrations. Exceeding these concentrations outside cells is toxic (exitotoxic) for neurons, leading to their death with a characteristic association with nonspecific permeability of mitochondria [122]. It should be noted that the level of glutamate is largely regulated by mitochondria, namely their ketoglutarate dehydrogenase [123], that is, glutamate production occurs inside cells.

By the way, the detoxification principle can be applied to the above-mentioned regulation by mitochondria of the level of fatty acids in the cell, which on the one hand can be uncouplers of oxidative phosphorylation, and on the other, oxidation substrates.

There is a point of view that mitochondria arose with the appearance of oxygen on earth, which has a rather significant and poorly regulated oxidizing ability, as a result of which cellular components could be oxidized, which is undesirable. Logically thinking, to limit such an undesirable process, it is enough to lower the intracellular oxygen concentration, and mitochondria could perform this function. Thus, mitochondrial oxidative activity can be considered as a special case of the detoxification process, and in relation to oxygen, mitochondria can be considered as fine regulators of its concentration in accordance with the oxygen affinity of mitochondrial cytochrome oxidase. The same can be attributed to ROS, the level of which in the cell is regulated by mitochondrial activity, subtly balancing the necessary production of ROS and their elimination, in particular, due to catalase [124], mitochondrial superoxide dismutase [125] or peroxidases [126-128]. In addition to enzymatic systems that regulate the levels of intramitochondrial and intracellular ROS, there is a whole set of low-molecular compounds in the cell that quench the high oxidative capacity of ROS, which can also be attributed to the detoxification system. This part will be briefly discussed in the next section concerning the redox buffer in the cellular system.

Creation of an intracellular redox buffer. Homeostasis of the redox potential in a cell is one of the bases of its healthy existence, and when it is disrupted, leading to temporary or chronic oxidative or regenerative stress, several pathological situations arise. It is known that almost all vascular pathologies of the heart, brain and kidneys associated with ischemic effects are the result of the fact that the cellular reserves of the redox buffer cannot cope with the oxidative challenge, which leads to oxidation of the vital components of the cell, requiring either internal repair or external intervention [15, 129-131].

As we discussed above, large capacities are concentrated in the cell and in the mitochondria to destroy excessive levels of ROS in the cell. They include, firstly, enzymes (superoxide dismutases, catalase, peroxidases, ferredoxin, etc.), which are designed to neutralize ROS, although not allowing an equally dangerous situation of ROS deficiency, which are an essential component of cellular metabolism. Secondly, these are small molecules united by the term antioxidants, the chemistry of which ensures the quenching of the high oxidative capacity of ROS. A quantitative assessment was given in the literature [132], but it concerned the gross antioxidant activity without specific highlighting the contribution of partial components.

Probably, the largest contribution to antioxidant activity is made by the proteins themselves, which carry groups capable of oxidation, for example, their sulfhydryle groups, capable of forming an S-S transition during oxidation. Of course, this process is highly undesirable in vital enzymes, given that such transitions will inevitably affect enzymatic activity. Apparently,

the evolutionary solution was to create proteins that do not carry obvious catalytic functions, but at the expense of mass acceptance of the oxidative threat. Obviously, this is the main function of mass proteins, such as albumins or structural proteins. Protein derivatives and peptides play a similar role. Admittedly, reduced GSH is the most important representative of such peptides, turning into the GSSG dimer upon oxidation. Although glutathione synthesis occurs in the cytoplasm, in the cell it is mainly contained in the mitochondria, where it is transported, creating the basis of the mitochondrial and cellular redox buffer.

In addition to glutathione, cytochrome *c*, localized in the intermembrane space, can play an antioxidative role in mitochondria, being associated with cytochrome oxidase [133] and mitochondrial contact sites [134, 135]. Besides the revealed peroxidase function of cytochrome *c* [136] due to its high abundance in the intermembrane space, the massive release of this protein from mitochondria during permeabilization of the outer membrane provides an intracellular concentration sufficient to consider its contribution to direct ROS quenching in the cell significant [137, 138]. Recently, we hypothesized that extended mitochondrial systems in the cell provide a more or less uniform distribution of redox potential in the cell [139]. It should be noted that for a number of years, the focus of V. P. Skulachev was in the development of his own concept of the functioning of extended mitochondrial systems as intracellular electrical cables [140-143].

Concluding this part, which describes the main vital functions of mitochondria, one can see how multidimensional is that part of the functioning of mitochondria that ensures the healthy existence of the cell thus leading to the conclusion that homeostasis of these vital functions is necessary. We did not review the participation of mitochondria in the processes of cell proliferation, differentiation, and thermogenesis, as well as avoided examining the important role of the mitochondrial genome, considering that these aspects were widely considered by other researchers, but were not included in the research scope of Skulachev and his closest colleagues.

MITOCHONDRIA AT THE CENTER OF DEATH

The death of a biological system implies the multilevel nature of such a specification, affecting the destruction of biological macromolecules (microphagy), cellular structures (macrophagy), cells (all types of cell death [144]), and organisms (phenoptosis). Each mechanism in these divisions requires an extended analysis, which, firstly, is not desirable within the framework of this brief review, and, secondly, given the focus of this work on the range of interests and works of V. P. Skulachev, we will limit ourselves to considering the participation of mitochondria in cell death and programmed death of the organism.

Mitochondrial and cellular death. By its name (translated from Greek μίτος – thread and χονδρίον – grain), mitochondria have long been considered as very labile structures existing in the form of extended filaments and small rounded structures, and this process naturally raised questions – why is there a constant generation of small structures that split off from the mother tree?

Probably, now an explanation can already be found for this. With a high probability, a constantly functioning electron transfer chain in mitochondria can be accompanied by electron leakage to various non-target components and lead to undesirable oxidation of these components. These oxidative damages may be repaired or not, and in the case of the latter, a scenario for the removal of these damaged components starts to emerge. In addition to oxidized components, improperly folded proteins and other biological structures modified by non-oxidative origin become unwanted for mitochondrion, which, by an unclear mechanism, begin to segregate within the same mitochondrion [145, 146]. This process of intramitochondrial separation of “correct” and “incorrect” elements is completed by their division by a membrane (septum), followed by separation of the damaged fragment and its disposal in the machinery of mitophagy. Under normal conditions, the number of detached small fragments is not too large, but under conditions of oxidative challenge, the entire mitochondriome undergoes the process of a total fission into fragments [147, 148], among which there may be preserved only a part that cannot be destroyed and which, if the oxidative challenge is eliminated, begins to serve as the basis for building a new mitochondriome due to fusion with other, uncritically damaged fragments. The described picture is somewhat speculative, but it has enough arguments supported by various data. In this whole scheme, the elimination (death) of the mitochondria, which is called “mitoptosis”, is mandatory for a healthy cell. A very important requirement for the initiation of mitochondrial death is oxidative stress, which, as it was found, can accompany the process of induction of nonspecific permeability in mitochondria [149-158]. This phenomenon, at the very beginning incomprehensible in its principle, was characterized by the induction of a megachannel in the inner mitochondrial membrane, which not only makes it impossible for the membrane potential and ion gradients to exist, but also leads to the high-amplitude swelling of mitochondria described above. The latter is accompanied by permeabilization of the outer mitochondrial membrane either due to its rupture caused by swelling [159] and/or due to

the organization of pores formed by heteromerization of the Bax protein [160]. Later, it has been suggested that the induction of nonspecific permeability of mitochondria is a step of programmed destruction of these organelles [161], and even later, this phenomenon was attributed to the point of no return, preceding programmed cell death, in particular, developing by the mechanism of necrosis [162]. The mitochondria are critical elements that determine their own fate and the fate of the cell, and the generally recognized concept of mitochondrial function as the determinant of the point of no return, that is, responsible for deciding whether or not to be a cellular system, puts the mitochondria at the center of a deadly cascade. To start this cascade, the mitochondria release a number of molecules (cytochrome *c*, AIF, procaspase IX, etc.), which alone, being inside the mitochondria, are not dangerous, but after interaction with the components of the cytosol, a complex is formed [163], which becomes a death sentence for the cell. This means that mitochondria formally carry reservoirs of cellular poisons, which inevitably cause cell death when appropriate signals enter the mitochondrion. Given the huge array of excellent reviews on this topic, we limit our discussion to the above.

Death of the organism (phenoptosis). In *Biochemistry (Moscow)* special issues devoted to the phenomenon of phenoptosis and in other publications, we have repeatedly written about the key role of mitochondria in this process [164-167]. The problem of phenoptosis, as a programmable mechanism for the death of a multicellular organism, for couple of last decades has been in the focus of Vladimir Skulachev, who introduced mitochondria and their ROS generation into the focus of this problem. There are several examples of phenoptic death and they can be found in the works of Skulachev [168-171]. It is assumed that phenoptosis was developed during evolution to reject unnecessary organisms, whether sick or aged, that is, all those that cannot compete with healthy and young organisms. There are two types of phenoptosis. One caused by stress, acute or rapid phenoptosis is a rapid deterioration of the organism's condition, subject to acute stress. Another type, caused by age, mild or slow phenoptosis, is characterized by a slow deterioration of the state, ending in the death of the organism due to the presence of chronic stress. It follows from this that aging and diseases associated with aging are hallmarks of phenoptosis. Skulachev himself believed that one of the best proofs of the involvement of mitochondria in the programmed death of the organism were experiments on the model of acute phenoptosis, in which animals having received an almost fatal sentence as a result of ischemia of the animal's only kidney, survived after the introduction of mitochondrial-directed cationic agents [172]. The fact that not all of these substances belonging to this group eliminated renal failure, from which a fatal outcome seemed to be expected, but they all saved from death, gave a strong evidence of the complex organization of the initiation of a deadly cascade, possibly remote from the target organ. This again indicated that it is the mitochondria that determine the general poisoning of the organism and the salvation of the latter is hidden in mitochondria. Of course, there were other experimental proofs of the accuracy of the participation of mitochondria in the death of the organism [173].

August Weismann is considered to be the founder of the theory of programmed death [174]. However, there is a point of view that the main ideas of programmed death of individuals were expressed earlier by Alfred Russel Wallace in his work Contributions to the Theory of Natural Selection, published in 1870, that was long before the discovery of mitochondria. We can safely go back and assume that the ideas that death is programmed were expressed even by the great Russian poet Alexander S. Pushkin, who wrote in 1828:

A random and a wasted gift
Is given life, I wonder why
By some and enigmatic shift
It always is condemned to die

(translation by D. B. Zorov)

The great poet, who at the time of writing these lines was not even 30 years old, wondered why life was condemned to death, that is, why it was inevitable. The great Russian scientist Vladimir Skulachev came to understand the high degree of organization of the deadly process. It allowed him to guess the basis of this mysterious process, which he wanted to stop, cancel and thus to prohibit the program of death of the organism, using knowledge of the role of mitochondria in the organization of vital and deadly processes.

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